Inactivation of bacteria in seafood processing water by means of UV treatment

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ABSTRACT

Seafood processing is a large-scale food industrial activity, in the UK and worldwide, which requires substantial quantities of clean water for washing purposes. Therefore, the aim of this study is to assess the feasibility of ultraviolet (UV) treatment to disinfect water coming from shellfish washing process, as to safely recycle it in the process. For this reason, different operating parameters that typically affect UV treatment efficiency, namely the power output of the UV lamp (5W, 9W, and 11W), the turbidity of the washing water (0 – 52 NTU), and the initial bacterial concentration (10^4, 10^5, 10^6 CFU mL^{-1}) were studied. Water disinfection was monitored by following changes in the concentration of the *Escherichia coli* (*E. coli*) bacteria. Photoreactivation of bacteria after UV disinfection was also investigated. Results showed that the UV treatment can efficiently inactivate bacteria in shellfish processing water, since *E. coli* (10^6 CFU mL^{-1}) in turbid (i.e. 0.074 – 35 NTU) seafood processing water were inactivated within the first 15 sec of treatment, by means of an 11 W germicidal lamp. Under these conditions, no bacteria photoreactivation was observed after 2 h of exposure to natural light. The disinfection efficiency was decreased when the initial bacterial concentration and water turbidity were increased. In addition, the increase of UV power output resulted in a substantial increase of bacterial inactivation. Furthermore, *E. coli* were reactivated after 2 h of exposure to natural light when the turbidity of the washing water was ≥ 42 NTU or when the initial bacterial concentration was high (i.e. 10^5 and 10^6 CFU mL^{-1}).

KEYWORDS: shellfish; seafood processing industry; water disinfection; aquaculture; water recycling

1. Introduction

Shellfish farming and packaging is a large-scale food industrial activity in the UK and worldwide. The UK exports most of the seafood it harvests, thus resulting to high economic gains (e.g. in 2011 just over 435,000 tonnes of seafood, worth £1.46 billion, were exported from the UK (Seafish, 2012a)). High value shellfish, such as langoustine, crab and scallops, are exported to the French, Spanish and Italian markets (Seafish, 2012a). Moreover, Scotland dominates the UK seafood processing industry, while secondary processing units are found in the North England and Wales, thus providing 11,864 full-time jobs in 325 units throughout the UK (data for 2011) (Seafish, 2012a, b). To maintain the high quality and profitability of the UK shellfish species, domestic suppliers have focused on improving the sustainability of their farming, as well as their packaging process.

Shellfish packaging requires vigorous washing and scrubbing with clean water, as to ensure maximum removal of sediments and other debris. Water should be taken from an appropriate source, which is usually sea or tap water (MassachusettsGeneralLaws, 2015). Nonetheless, seawater pumping is an energy intensive process, while also it may be inappropriate due to high pollution levels. It has been extensively reported that seawater in the European continent and worldwide face great challenges due to heavy metal (Besada et al., 2011; Kallithrakas-Kontos and Foteinis, 2015; Wang et al., 2013) and oil pollution (Cohen, 2013). Moreover, many seafood processing industries are sited inland, therefore seawater utilization is unpractical. In these cases, tap water is the only solution, but its use can significantly increase operational costs and negatively affect the sustainability of the process. Furthermore, shellfish processing machinery consumes large amounts of water (e.g. for shellfish washing, equipment and floor cleaning), while water reclamation and recycling is not applied. Therefore, water minimization and reuse strategies should be introduced in such industries, as to make seafood washing more efficient and sustainable; thus improving their overall environmental footprint, competitiveness and profitability.

Ultraviolet (UV) irradiation is a well-established treatment technology for bacterial inactivation in water, air and solid surfaces and is one of the approved technologies used for food processing and preservation (EPA, 2006; Gardner and Shama, 1999; Quek and Hu, 2013; Venieri et al., 2013). The efficiency of UV treatment is attributed to the hazardous effects of UV-C radiation, which can destroy directly the DNA and the outer cell membrane of pathogenic microorganisms (Chatzisymeon et al., 2011; Venieri et al., 2013). UV-C irradiation between 250 nm and 270 nm, where the maximum absorbance of nucleotide bases

of the genome occurs, including thymine, cytosine and uracil, can induce damages in DNA and RNA, thus inhibiting cell transcription and replication (Vélez-Colmenares et al., 2012). Specifically, the major DNA lesion, induced by germicidal UV-C irradiation at 254 nm, is the formation of pyrimidine dimers. The presence of these lesions inhibits the normal replication of DNA, and therefore results in inactivation of the microorganisms in short time periods (Nebot Sanz et al., 2007). In addition, UV disinfection does not require chemical reagents, thus another advantage is that there is no formation of hazardous disinfection by-products after treatment (Summerfelt, 2003). However, its main drawback is that many microorganisms, including bacteria, are known to possess the ability to repair their DNA damage in the presence (photoreactivation) or absence (dark repair) of light (EPA, 2006; Nebot Sanz et al., 2007; Quek and Hu, 2013; Sinha and Hader, 2002). This can lead to the reactivation of bacteria, after UV treatment, hence affecting disinfection efficiency and rendering UV treatment unsafe. Till now, few studies have dealt with the use of UV irradiation for food processing, including the inactivation of bacteria on raspberries and strawberries (Bialka et al., 2008), in fruit juices (Gayán et al., 2012; Müller et al., 2011; Santhirasegaram et al., 2015), apple cider (Unluturk et al., 2004), goat milk (Kasahara et al., 2015), and in liquid egg products (Unluturk et al., 2008). However, to the best of the author's knowledge, there is no study dealing with the application of UV for the treatment of seafood processing waters.

Therefore, the aim of this study is to investigate the feasibility of the UV method to disinfect shellfish washing water, thus being able to safely recycle treated water in the process. For this purpose, washing water from a shellfish processing industry was used and various operating parameters that typically affect UV efficiency were studied. These were the lamp power output, the initial bacterial concentration, water turbidity and treatment time. The effect of bacterial photoreactivation on treatment durability was also examined, as to ensure the feasibility of the process.

2. Materials and Methods

2.1. Shellfish processing water

Shellfish processing water was collected from an industry that uses tap water for shellfish washing, located in the UK. The processing water originates from the industry's shellfish

94 washing line, where tap water is initially used and then it is collected in tanks (about 250 L)
95 and reused, if appropriate, in the washing process. However, shellfish-associated bacteria,
96 including potential pathogens and spoilage organisms, build up in the tanks, thus rendering
97 the used water inadequate for recycling purposes after a short period of time. Therefore, this
98 water has to be disposed of, about every 10 min, when the bacterial concentration becomes
99 too high. thus preventing the efficient water recycling in the washing stage, and fresh tap
100 water needs to be introduced in the system. Shellfish-associated bacteria can include *Vibrio*
101 and *Shigella* species, *Salmonella*, or other toxin-forming bacteria (Iwamoto et al., 2010). In
102 this work, water disinfection was monitored by following changes in the *E. coli* bacteria,
103 which is a common and very popular indicator pathogenic microorganism for potable water
104 (Chatzisymeon et al., 2011), since according to current legislation the quality of seafood
105 washing water should follow the standards of drinking water (MassachusettsGeneralLaws,
106 2015).

107 In order to measure bacterial contamination in the used washing water and assess the
108 feasibility of the UV treatment, tap water was continuously (i.e. every 10 min) recycled in the
109 shellfish washing line for up to 40 min. Washing water samples were withdrawn after 10, 20,
110 30, and 40 min of washing, as to measure their physicochemical and microbiological
111 characteristics. The water samples were collected in sterilized sampling bottles of 1 L, kept at
112 4 °C and immediately dispatched for further analyses. After measuring their characteristics,
113 samples were sterilized at 121 °C for 15 min and kept in the fridge (4 – 8 °C).

114

115 **2.2. Bacterial strain**

116 The bacterial strain of *Escherichia coli*, which was used in this work as a water quality
117 indicator, was isolated from the shellfish washing waters by membrane filtration. From the
118 collected samples 200 μL were passed through a 0.45 μm pore-sized filter (cellulose
119 acetate/nitrate membranes by Sigma-Aldrich), using a vacuum pump VP series (KNF Lab).
120 These membranes were aseptically placed up on plates with Brilliance *E. coli*/Coliform Agar
121 (Oxoid) selective media, thus ensuring that no air bubbles were trapped. The plates were
122 incubated at 37 °C for 20 – 24 hours and *E. coli* colonies with purple-blue colour were picked
123 for further use. Specifically, the isolated *E. coli* were spiked into the sterile industrial washing
124 water to achieve the desired initial bacterial loading for each experimental run. The standard
125 *E. coli* ATCC 23716 (American Type Culture Collection, Rockville, MD, USA) strain was

also used. The freeze-dried cultures were rehydrated and reactivated according to the manufacturer's instructions. The concentration of bacterial cells in the shellfish processing water ranged from 10^4–10^6 CFU mL^{-1}, as estimated by measuring its optical density at 600 nm on a Cary100 UV-Vis double-beam (Varian, Inc.) spectrophotometer.

2.3. UV experiments

Experiments were conducted in an immersion well, batch type, laboratory scale photoreactor shown in Schematic 1. This is a two compartment apparatus and consists of an inner quartz glass housing the lamp and an exterior cylindrical reaction vessel made of borosilicate glass. The reaction mixture was placed in the exterior cylindrical reaction vessel (compartment 1) and the inner quartz glass was immersed inside the reaction mixture. The UV lamp was placed inside the inner glass tube (compartment 2). It should be noted that this apparatus was constructed and assembled in the workshop of the University of Edinburgh, UK. In a typical experimental run, 300 mL of the shellfish processing water were introduced in the reaction vessel. The bacterial suspension was magnetically stirred, to ensure complete mixing of *E. coli* with the processing water, and then the UV lamp was turned on. UV-C irradiation, with emission wavelength at 254 nm, was provided by an 11 W (11TUV, PL-S, Philips) or a 9 W (PL, 2 PIN, Philips) or a 5 W (5TUV, PL-S, 2G7 base, Philips) germicidal lamp. The temperature was constant at 18 ± 1 °C (i.e. ambient temperature), during each experimental run, since in the shellfish processing industry the washing process takes place at ambient conditions. The exterior reaction vessel was covered with aluminium foil to reflect back UV irradiation. Representative experiments were carried out in triplicates to check the reproducibility of the process. At specific time intervals, 2 mL of the reaction solution were withdrawn and immediately analysed with respect to viable *E. coli* cells, by the serial dilution culture method.

Schematic 1

2.4. Microbiological and chemical analyses

155 The detection and quantification of *E. coli* in the processing water was performed using the
156 serial dilution pour plate agar technique. Serial dilutions of the reaction solution were
157 performed in sterile 0.8% (w/v%) NaCl (Fisher Scientific, UK) aqueous solution and 200 μL
158 of each dilution (including neat sample) were pipetted and spread onto Brilliance *E.*
159 *coli*/Coliform Agar (Oxoid) plates, a selective culture medium. The plates were incubated at
160 37 ◦C for 20–24 h before viable counts were determined. *E. coli* colonies appeared with
161 purple colour, while coliforms colonies had a pinkish colour. For the undiluted samples, 1
162 mL of sample was spread over five 90 mm Petri dishes (i.e. 200 μL of sample per Petri dish).
163 This was done to reduce the detection limit to 1 CFU mL^{-1} for the undiluted samples
164 (Paleologou et al., 2007; Rincón and Pulgarin, 2004).

165 The turbidity was measured on a HACH 2100N turbidity meter, while conductivity and pH
166 were measured by a portable conductivity and pH meter (± 0.1 pH accuracy), respectively, by
167 Hanna Instruments.

168

169 **2.5. Photoreactivation experiments**

170 Bacteria are known to be capable of repairing their damaged DNA after UV treatment, either
171 by dark repair or by photoreactivation mechanisms (Chatzisymeon et al., 2011; Venieri et al.,
172 2011). The latter is considered to be the most important mechanism (Nebot Sanz et al., 2007).
173 In addition, in the seafood processing industry, under study, the tanks, where the shellfish
174 washing water is collected and it is then recycled into the washing process, are open and
175 exposed to natural light. Therefore, in this case, the investigation of bacterial
176 photoreactivation is of major importance. Most photoreactivation studies involve the use of
177 visible light from artificial sources, such as fluorescent lamps, which emit light at 360 nm and
178 halogen lamps emitting between 400 nm and 800 nm. However, very few have dealt with
179 natural light (Chatzisymeon et al., 2011; Vélez-Colmenares et al., 2012; Venieri et al., 2011),
180 as is the case of the present work. Specifically, *E. coli* photoreactivation experiments in UV
181 treated shellfish washing water were carried out under natural light. For this reason, 100 mL
182 of the final treated effluent were transferred into a sterile conical flask, which was then sealed
183 up to prevent air getting in and potentially contaminating the effluent. The flasks were kept
184 under continuous stirring for about a day (22 h) and under natural light conditions. After this
185 period the final sample was analysed in terms of *E. coli* viability.

3. Results and Discussion

3.1. Physicochemical and microbiological characteristics of the shellfish washing water

The physicochemical and microbiological characteristics of the collected washing water are shown in Table 1, where it can be observed that conductivity is increasing with washing time, from the initial value of 0.05 to 0.52 mS/cm, after 40 min of shellfish washing. This increase in conductivity can be attributed mainly to the increased water salinity, deriving from dissolved salts coming out from shellfish washing. Interestingly, turbidity is increased from 0.079 to 42.7 NTU during the first 10 min of washing; while further washing (i.e. from 10 min to 40 min) does not considerably affect turbidity. This sharp increase of turbidity from the first 10 min of washing is attributed to solid particles that are washed out from the shellfish; these may include cracked shells, seaweed residuals, etc.. Moreover, turbidity values remained at the same order of magnitude for the rest of the washing time, e.g. 42.7 NTU at 10 min to 52 NTU at 40 min. Although, it was expected that water turbidity would be rapidly increased, due to the high loads of solids, which are washed out during the washing process, this is not the case here. This can be attributed to the fact that a sieve to hold all large solid particles coming out of the washing process was installed at the end of the shellfish washing line, and therefore this is the main reason that turbidity is increased up to a value of about 42 – 52 NTU and after that it remains almost constant with time. Finally, a slight increase of pH values by time is also observed, which can be attributed to the increase of conductivity and turbidity. Conductivity (i.e. content of salts in water) and turbidity (i.e. suspended solids coming from cracked shells and residual seaweeds) can have neutral or alkaline pH values, thus slightly increasing the pH of the washing water from 5.76 to 6.14.

Table 1.

As far as the microbiological characteristics are concerned, it was observed (Table 1) that pathogen microorganisms, namely *E. coli* along with other coliforms, were increased up to the order of 10^3 and 10^4 CFU mL^{-1}, respectively, after 20 min of washing. Surprisingly, further processing did not cause any greater increase of bacterial concentration in the washing

water. This can be explained by the increased (≥ 0.42 mS/cm) conductivity (i.e. salinity) of the water, which prevented the further growth of bacteria in water (Kaspar and Tamplin, 1993). In general, enteric bacteria, when released into saline water, are subjected to an immediate osmotic shock, and their ability to overcome this by means of several osmoregulatory systems could largely influence their subsequent survival in the marine environment (Rozen and Belkin, 2001). Specifically, the survival of *E. coli* bacteria in saline water depends, at least partly, on whether they possess certain genes which enable them to regulate osmotic pressure and whether they can be stimulated to express those genes before or after their release into the saline aquatic environment (Munro et al., 1989). For example, in a previous study it was observed that survival of *E. coli* in seawater/distilled water mixtures at different ratios (0, 25, 50, 75 and 100% seawater) for 48 h showed an optimal survival (74%) at the 25% seawater mixture (Carlucci and Pramer, 1960). Moreover, Anderson et al. (1979) who studied the survival of an *E. coli* isolate for 8 days in seawater at selected salinities (1, 1.5, 2.5, and 3%), observed that decreasing salinity was accompanied by increasing survival (Anderson et al., 1979). Finally, the slight decrease in bacteria counts (Table 1) from 30 min to 40 min of washing can be assumed as negligible, since this is within the same logarithmic order of magnitude.

3.2. Effect of UV power

The effect of UV power on inactivation of bacteria was also studied. For this purpose, three UV lamps, with different power outputs of 5 W, 9 W, and 11W, were used. It should be noted that, in this case, turbidity can be assumed as constant, since there is a similar effect on disinfection efficiency when turbidity values are ≥42 NTU (see section 3.4). Results are shown in Figure 1, where it is observed that the inactivation of bacteria is rapidly increasing with increasing the power output. Thus, the 11 W UV lamp achieved total inactivation of bacteria after 30 sec of treatment, which was not the case for either the 5 W or the 9 W lamp. Specifically, when initial bacterial concentrations of the order of 10^6 CFU mL^{-1} are concerned, the 5 W and 9 W germicidal lamps did not achieve water disinfection, not even after 4 min of treatment. In general, photolysis in real water samples occurs directly through light absorption by the organic molecules of the bacterial cells (Chatzisymeon et al., 2011; Nebot Sanz et al., 2007; Vélez-Colmenares et al., 2012; Venieri et al., 2013). Therefore, the higher performance of the 11 W UV system can be attributed to the higher photon flux that

finally reaches the reactant solution and causes the rapid photolytic degradation of bacteria. In addition, the treatment time obtained here is comparable with previous studies, where *E. coli* inactivation in biologically treated municipal effluents occurred after 3 min of UV irradiation with an 11 W germicidal lamp (Chatzisymeon et al., 2011). It should be noted that experiments were performed with initial bacterial concentration of 10^6 CFU mL^{-1}, which is above the real bacterial concentration (i.e. 10^4 CFU mL^{-1}), as shown in Table 1. This was done to ensure that UV treatment can work under stressed (high bacterial load) conditions. Summing up, a UV germicidal lamp with power output ≥11 W can become a feasible option for disinfecting shellfish processing washing waters, thus improving the overall sustainability of the industrial process.

Figure 1.

3.3. Effect of bacterial concentration

The effect of bacterial initial concentration on process efficiency was investigated and the results are presented in Figure 2. Three different initial bacterial concentrations, i.e. 10^4, 10^5, and 10^6 CFU mL^{-1}, were tested; which are substantially above the *E. coli* loadings (i.e. 10^3 – 10^4 CFU mL^{-1} as shown in Table 1) in real washing waters. It was observed that inactivation of bacteria occurs more rapidly when their initial concentration is lower. For example, when the initial *E. coli* concentration was 10^4 CFU mL^{-1}, water was disinfected after 240 sec of treatment, while for initial concentration of 10^6 CFU mL^{-1}, a substantial amount of *E. coli* (10^3 CFU mL^{-1}) survived after 240 sec of treatment. Results in Figure 2 show that the amount of photons emitted from the 9 W germicidal lamp were not adequate to disinfect *E. coli* of 10^5 and 10^6 CFU mL^{-1} initial concentrations, within the first 240 sec of treatment. On the other hand, for initial bacterial concentrations of $\leq 10^4$ CFU mL^{-1}, results show that the 9 W germicidal lamp can be a feasible and applicable option for shellfish processing water disinfection and recycling. Nonetheless, since initial bacterial concentrations are not always $\leq 10^4$ CFU mL^{-1}, disinfection cannot at all times be secured in shellfish processing water, and therefore a germicidal lamp of 11 W, or higher, is proposed as a feasible alternative for recycling shellfish washing water.

279 Figure 2.

280

281 **3.4. Effect of water turbidity**

282 Water turbidity is a parameter that can negatively affect the efficiency of UV treatment, and
283 thus its investigation is of major importance. Process efficiency may be inhibited by the
284 presence of suspended solids in the water (Gullian et al., 2012). Inhibition is mainly
285 attributed to the facts that (a) turbidity prevents light from penetrating the whole water
286 matrix, and (b) bacteria can be shielded by solids, thus protecting them from exposure to UV
287 light and therefore preventing their inactivation. Therefore, a series of experiments was
288 performed to assess the effect of water turbidity on process efficiency. The range of turbidity
289 that was examined corresponds to the ones observed in the real shellfish washing water, i.e.
290 35 – 52 NTU, and the results are shown in Figure 3. It can be observed that for turbidity
291 lower than 35 NTU, bacteria are rapidly inactivated after the first 15 sec of treatment. When
292 turbidity values are 42.7 NTU and 52 NTU, results show that *E. coli* appear to have been
293 inactivated during the first 2 min of UV treatment. However, there is a bacterial increase to
294 15 CFU mL^{-1} after 4 minutes of treatment and this reappearance can be explained by the fact
295 that the high turbidity of the water (i.e. 42.7 and 52 NTU) can both shield bacteria and hinder
296 the penetration of UV irradiation into the whole liquid volume, thus preventing its effective
297 disinfection. Therefore, it is highly recommended that turbidity should be decreased (≤ 35
298 NTU) before UV treatment, as to optimize the treatment time and process efficiency.

299

300 Figure 3.

301

302 **3.5. Effect of bacterial strain**

303 All the aforementioned experimental series were carried out by spiking *E. coli*, initially
304 isolated from the fresh shellfish washing water, into the same matrix, as to obtain the
305 desirable initial bacterial concentration. In order to confirm and generalize the feasibility of
306 the UV treatment for disinfecting such type of waters, experiments were also performed by
307 using the standard *E. coli* strain ATCC 23716. Results are shown in Figure 4, where it is
308 evident that the disinfection efficiency of the standard strain is slightly higher, than in the

case of the bacteria isolated from the real environmental samples. This indicates that isolated bacteria are more persistent to UV treatment than standard strains, thus highlighting the importance of this work which deals with the inactivation of isolated bacteria in real industrial shellfish washing waters. There can be many causes for the difference in the resistance of *E. coli* bacteria to UV treatment. Firstly, during evolution these are possibly exposed to various kinds of environmental stresses, such as temperature, water medium, UV irradiation or chemical agents. Each of these stresses can act differently on the bacterial cell and cause lethality that can vary from strain to strain (Chintagari et al., 2015). Moreover, UV is absorbed by nucleic acids producing several types of damage that interfere with replication and transcription of DNA. If UV-induced damage is not repaired or eliminated from DNA, it may lead to mutagenesis and cell death. Mutations not only promote genetic divergence of populations living in different environments, but even in identical environments parallel populations may diverge, if they find alternative adaptive solutions. To prevent the lethal effects of this and other DNA damaging agents, different repair mechanisms have developed through evolutionary history. Therefore, during adaptation of *E. coli* to UV irradiation, mutations induced in DNA repair or replication genes can be indiscriminately selected (Alcántara-Díaz et al., 2004; Chintagari et al., 2015).

Figure 4.

3.6. Photoreactivation of bacteria

Bacterial photoreactivation experiments were carried out, as to determine the efficiency of UV treatment. At the premises of the seafood processing industry under study, shellfish washing water is exposed to visible light before its further use. Thus the investigation of bacterial photoreactivation is imperative in order to ensure the safe UV treated water recycling supply. The results are shown in Table 2 and it is shown that in all cases *E. coli* photoreactivation occurs after 22 h of exposure to natural light. However, no reactivation was recorded after exposure to light for 2 h, at low initial bacterial concentration (i.e. 10^4 CFU mL^{-1}) (Run 1, Table 2), at low turbidity value of 35 NTU (Run 5, Table 2), and during the treatment of standard *E. coli* strains (Run 7, Table 2). Therefore, these results indicate that UV-C irradiation can cause severe damage to bacterial cells. Comparing the effect of initial

bacterial concentration on photoreactivation (Runs 1 – 3), it is observed that in cases where the initial *E. coli* concentrations are high, i.e. 10^5 CFU mL^{-1} and 10^6 CFU mL^{-1}, photoreactivation takes place after only 2 h of exposure to natural light. This shows that when increasing the initial bacterial concentration at 10^5 CFU mL^{-1} and above, photoreactivation is favoured. It should be noted that, in this case, turbidity can be assumed as constant, since as it was proved in section 3.4 there is a similar effect on disinfection efficiency, when turbidity values are ≥42 NTU. Moreover, as shown in runs 3, 4, and 6, photoreactivation is not affected by the different UV doses and occurs at all UV power outputs (11, 9, and 5 W). This is in contrast with previous studies, where it was observed that an increase in UV dose is valuable in minimizing photoreactivation events, since reduced UV dose causes reduced DNA damages on targeted bacteria, thus increasing the risk of subsequent photoreactivation (Lindenauer and Darby, 1994; Nebot Sanz et al., 2007). However, in this case it should be noted that runs 3, 4, and 6 are carried out at high turbidity values (i.e. 42 – 52 NTU) that has been proved to decrease disinfection efficiency. Not only this but, if runs 5 and 6 are compared, it is observed that at low turbidity values (i.e. 35 NTU) photoreactivation of bacteria does not occur for at least 2 h after UV treatment, while when turbidity is 52 NTU (run 6) photoreactivation takes place within the first 2 h after UV treatment. Furthermore, from runs 5 and 7, it can be concluded that bacterial strain has an effect on photoreactivation of *E. coli*, since, although both strains were reactivated after 22 hours of exposure, the cell count was higher for isolated bacteria. This is consistent with the results described in Figure 4 and enhances the fact that isolated bacteria are more resistant to UV treatment than standard strains, such as the ATCC 23716, which also highlights the significance of this work.

Table 2.

4. Conclusions

In this work the feasibility of UV treatment to disinfect shellfish processing water was assessed. For this purpose, the effect of important operating parameters, such as the initial bacterial concentration, UV power output, water turbidity and treatment time, on process efficiency was investigated. It should be noted that although this is a pressing problem for

seafood industry, it has received very little attention till now. The main findings of this work can be summarized as follows:

- Shellfish washing waters are turbid and saline with values ranging between 35 – 52 NTU and 0.28 – 0.52 mS/cm, respectively. Regarding their microbiological characteristics, there is a built-up of *E. coli* and other coliforms of the order of 10^3 CFU ml^{-1} and 10^4 CFU mL^{-1}, respectively.
- UV treatment can be efficiently applied to disinfect shellfish washing water, since it was observed that, at optimal operating conditions (i.e. UV power output at 11 W, water turbidity $\leq$ 35 NTU and initial *E. coli* concentration up to 10^6 CFU mL^{-1}) the total inactivation of bacteria is achieved after only 15 sec of treatment.
- Bacterial photoreactivation experiments were carried out and showed that no *E. coli* photoreactivation occurs, after exposure to light for 2 h, at low initial bacterial concentration (i.e. 10^4 CFU mL^{-1}), at low turbidity value of 35 NTU, and during the treatment of standard *E. coli*. Hence, it can be concluded that UV disinfection of shellfish washing waters, with initial bacterial loading of up to 10^4 CFU mL^{-1}, can be a very efficient treatment process in the presence of a UV lamp with power output of 11 W and when turbidity of the washing water is decreased to $\leq$ 35 NTU.

Acknowledgements

This research was funded by a grant from Interface Food and Drink (IFD).

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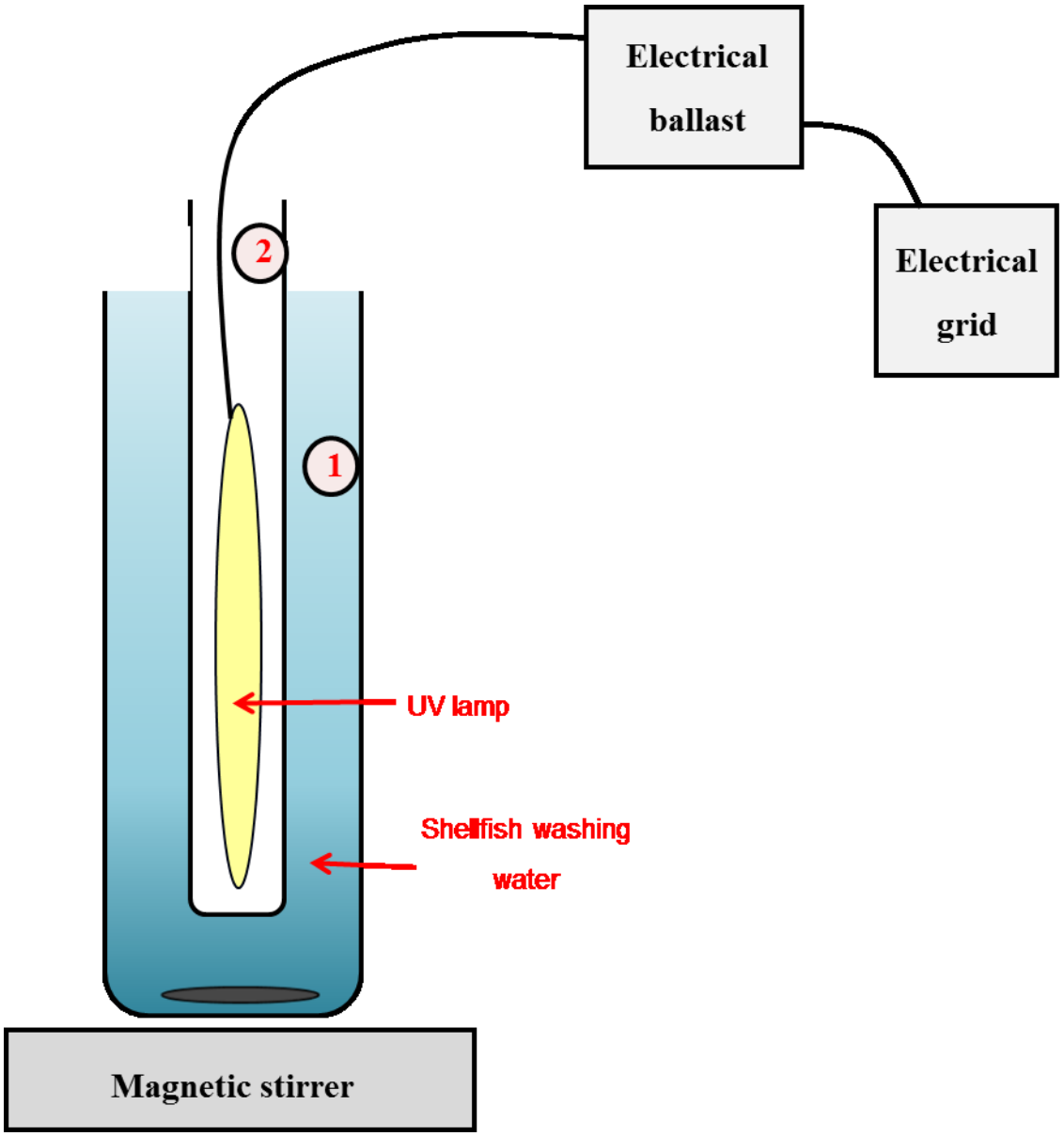
List of Schematics

Schematic 1. Experimental set up of the UV reactor. Compartments: (1) exterior cylindrical reaction vessel made of borosilicate glass, and (2) inner quartz glass tube housing the lamp.



Schematic 1. Experimental set up of the UV reactor. Compartments: (1) exterior cylindrical reaction vessel made of borosilicate glass, and (2) inner quartz glass tube housing of the lamp.

499 **List of Tables**

500

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506

507 Table 1. Physicochemical and microbiological characteristics of shellfish washing water
508 samples. The standard deviation (SD) is shown in brackets.

Characteristics	**Sample (shellfish washing time)**				
	1 (0 min)	**2 (10min)**	**3 (20 min)**	**4 (30 min)**	**5 (40 min)**
Conductivity, mS/cm	0.05	0.28	0.42	0.46	0.52
pH	5.76	5.87	5.98	6.2	6.14
Turbidity, NTU	0.079	42.7	42	35	52
***Escherichia coli*, CFU mL^{-1}**	0 (SD=0)	510 (SD=14)	1235 (SD=230)	7530 (SD=1010)	2420 (SD=380)
Coliforms, CFU mL^{-1}	0	750	20000	33583	15375

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512 Table 2. *E. coli* photoreactivation, under natural light, in UV treated shellfish processing
513 water.

Operating conditions of UV treatment					***E. coli* survival after 240 sec of UV treatment, CFU mL^{-1}**	***E. coli* survival after 2h of phototreatment, CFU mL^{-1}**	***E. coli* survival after 22h of phototreatment, CFU mL^{-1}**
Run	**Lamp power, W**	**Turbidity, NTU**	**Initial *E. coli* concentration, CFU mL^{-1}**	**Bacterial strain**			
1	**9**	**52**	**10^4**	**Isolated**	0	0	100
2	**9**	**52**	**10^5**	**Isolated**	57	18	>100
3	**9**	**42**	**10^6**	**Isolated**	2600	>100	>100
4	**5**	**42**	**10^6**	**Isolated**	7500	>100	>100
5	**11**	**35**	**10^6**	**Isolated**	0	0	>100
6	**11**	**52**	**10^6**	**Isolated**	15	>100	>100
7	**11**	**35**	**10^6**	**ATCC23716**	0	0	20

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516 **List of Figures**

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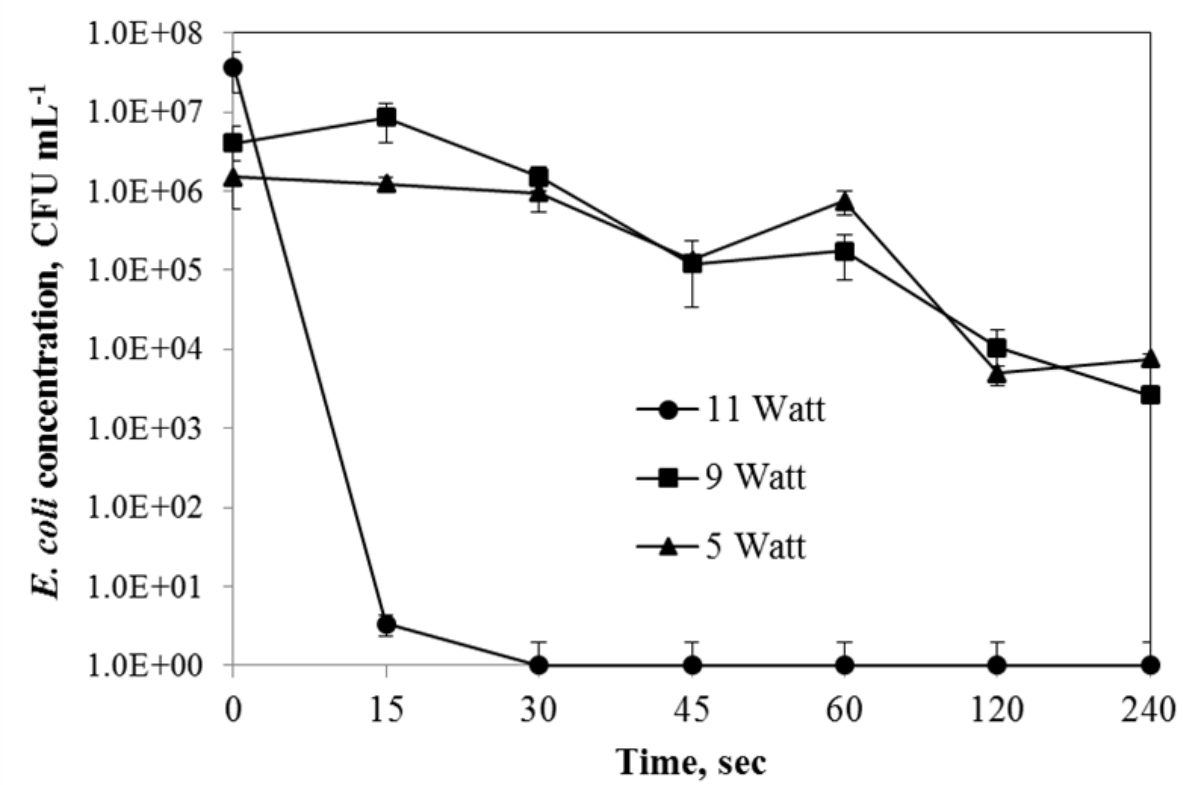
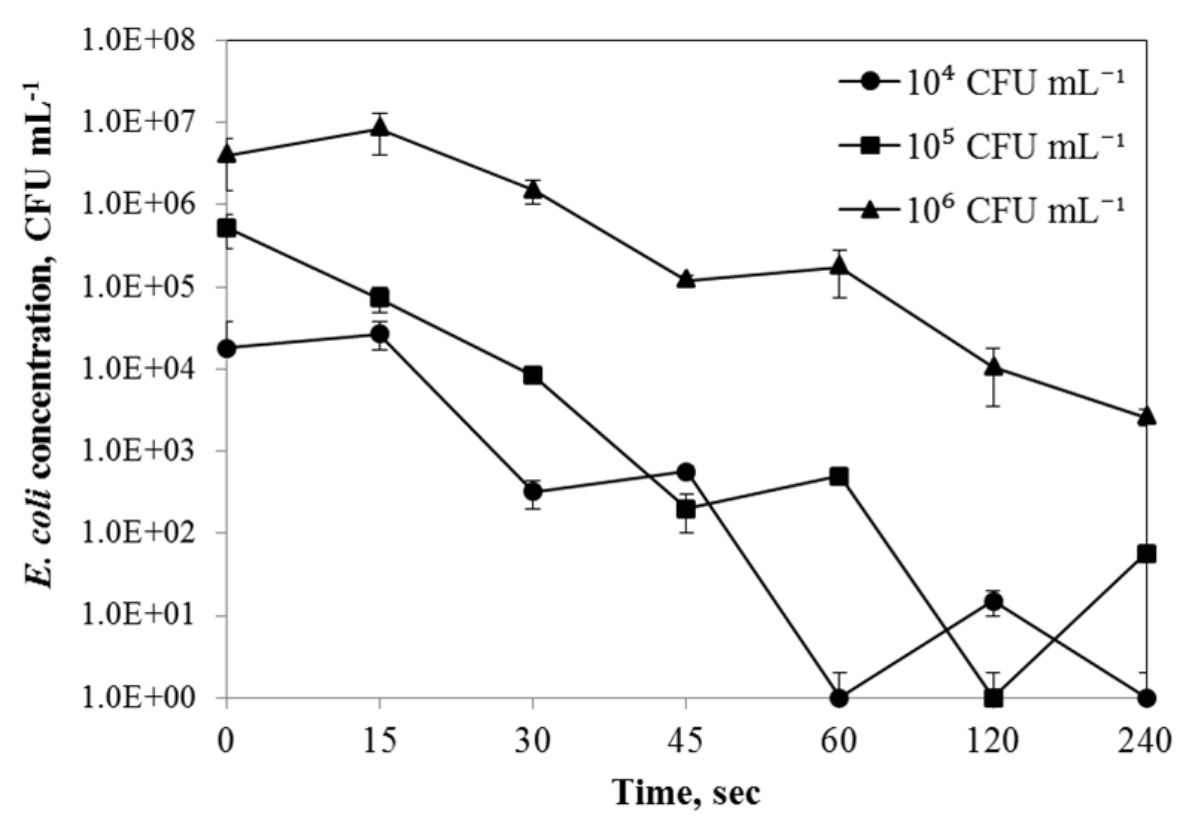


Figure 1. Inactivation of bacteria under different UV power outputs. Conditions: Initial bacterial concentration = 10^6 CFU mL^{-1}; water turbidity = 42 – 52 NTU.



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535 Figure 2. Inactivation of bacteria under different initial bacterial concentrations. Conditions:
536 UV power = 9 W; water turbidity = 52 NTU.

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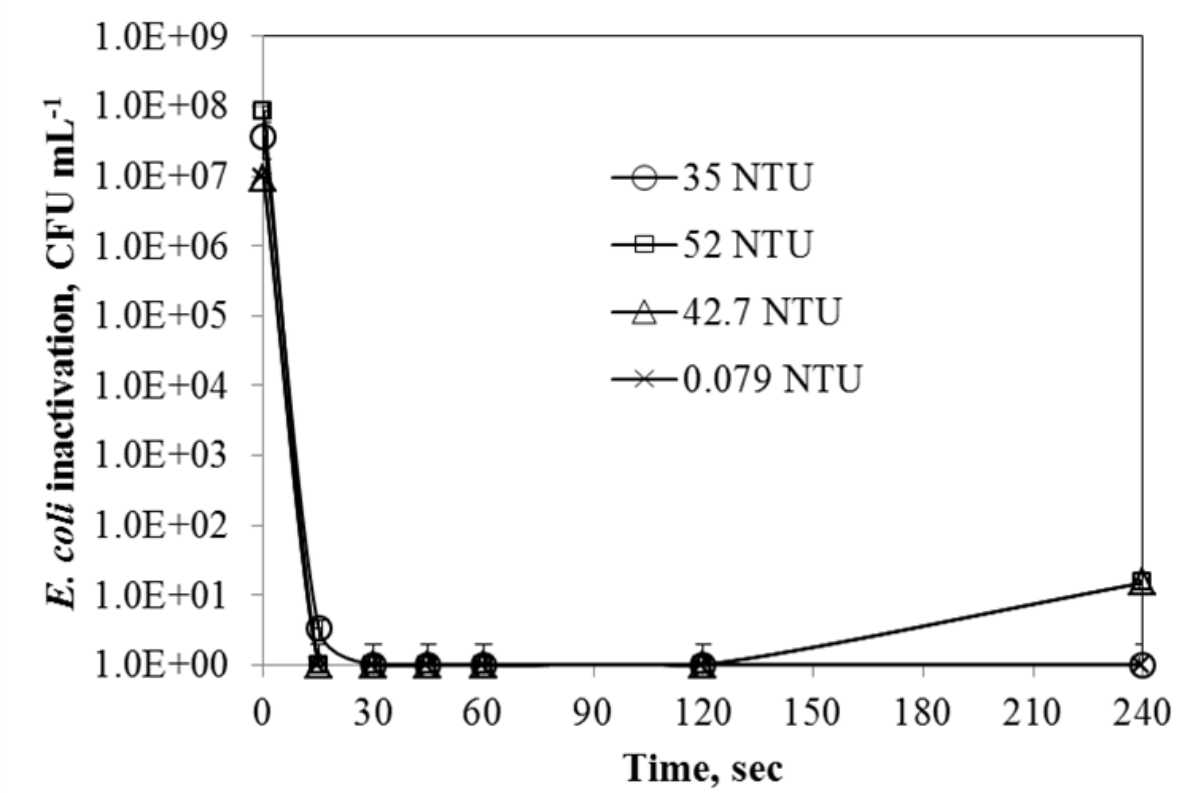

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539 Figure 3. Inactivation of bacteria under different water turbidity values. Conditions: UV
540 power = 11 W; initial bacterial concentration = 10^6 CFU mL^{-1}.

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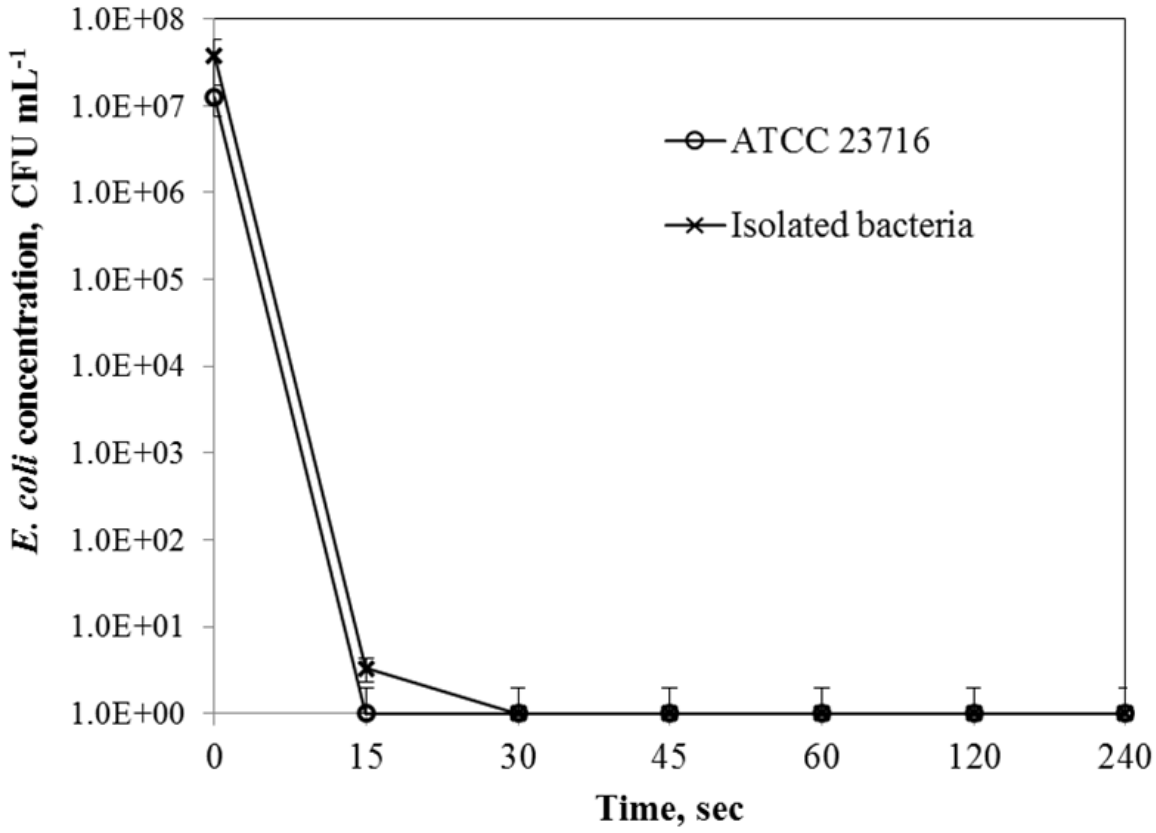

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544 Figure 4. Inactivation of bacteria in the presence of different *E. coli* strains. Conditions: UV
545 power= 11 W; initial bacterial concentration = 10^6 CFU mL^{-1}; water turbidity = 35 NTU.